APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1984, p. 879–881
0099-2240/84/040879-03$02.00/0

Vol. 47, No. 4

Effect of Relative Humidity on the Airborne Survival of Rotavirus SA11

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Received 28 December 1983/Accepted 26 January 1984

Rotavirus SA11, suspended in tryptose phosphate broth with 2.5 mg of rhodamine B per ml, was aerosolized (Collison nebulizer) into a rotating drum, and the aerosols were held at 20 ± 1°C with the desired relative humidity (RH). An all-glass impinger with tryptose phosphate broth was used to collect 1-min (5.6-liter) samples of air from the drum. The virus was found to survive best at medium (50 ± 5%) RH, where its half-life was nearly 40 h. The half-life of the virus at the low (25 ± 5%) RH level was about 9 h. Even at 72 h of aerosol age, 45 and 21% of the infectious virus remained detectable in the air at the medium and low RH levels, respectively. The high (80 ± 5%) RH level was found to be the least favorable to the survival of the virus, since 50% of the infectious virus became undetectable within 2 h of aerosolization. In a separate experiment at the midrange RH, 3% of the infectious virus was detectable in the drum air after 223 h (9 days) of aerosol age. Rotaviruses could, therefore, survive in air for prolonged periods, thus making air a possible vehicle for their dissemination.

Members of the rotavirus group are now well recognized as important pathogens of humans as well as a variety of animals (6). Apart from cases of rotaviral diarrhea in the general community, outbreaks due to these viruses frequently occur in hospitals (18). Such outbreaks have also been reported in nursing homes (3, 10), day-care centers (25), and schools (11). The exact mechanisms of virus spread during these outbreaks are not yet fully clear. However, air is suspected as a vehicle in their direct or indirect spread (7, 13). Before detailed epidemiological studies could be attempted to substantiate this, it was considered important to find out how well rotaviruses could survive in the airborne state.

The MA-104 cell line was used throughout this study. The procedures for the cultivation, maintenance, and passage of these cells have been described in detail previously (26). Cell cultures for virus plaque assay were put up in 12-well plastic plates (Costar, Cambridge, Mass.). Each well was seeded with ca. 5×10^4 cells in 2.0 ml of minimal essential medium in Earle base (Autopow; Flow Laboratories, Inc., Rockville, Md.) with 10% fetal calf serum (Flow). The plates were sealed individually in plastic bags (Phillips Electronics Ltd., Toronto, Canada) before being placed at 37°C in an ordinary walk-in incubator. The monolayers were generally ready for plaque assay within 48 h of seeding.

Simian rotavirus SA11 (strain H-96) was first plaque purified in MA-104 cells, and the same cells were used for the preparation of virus pools. To ensure that the pools contained only monodispersed particles of the virus, they were filtered through a polycarbonate membrane (Bio-Rad Laboratories, Richmond, Calif.) with a pore diameter of 80 nm. The plaque assay procedure has been described in detail previously (27).

The virus was aerosolized with the help of a six-jet Collison nebulizer (17) purchased from BGI Inc., Waltham, Mass. A 300-liter stainless steel drum (9, 21) was used for the storage of the aerosols. The drum was rotated at 4 rpm to reduce loss of the aerosols by sedimentation (9). The temperature inside the drum was kept at 20 ± 1°C. Drum air containing the virus aerosols was sampled at appropriate intervals with an all-glass impinger (34). A critical vacuum was maintained in all experiments so that the impinger would operate at its design capacity of 5.6 liters/min.

Rhodamine B (Eastman Kodak, Rochester, N.Y.), a fluorescent dye, was used as a physical tracer (5, 30) in this study. Reference solutions for the standardization of the dye were prepared in tryptose phosphate broth. An Aminco-Bowman spectrophotofluorometer (American Instrument Co., Silver Spring, Md.) was used for measuring dye concentrations in the samples. The excitation and emission wavelengths used were 546 and 590 nm, respectively.

The spray fluid consisted of tryptose phosphate broth with 2.5 mg of rhodamine per ml and Antifoam C (Sigma Chemical Co., St. Louis, Mo.) at a final concentration of 1%. The Collison nebulizer, with 15 ml of the appropriate spray fluid, was attached to the inlet of the rotating drum, and aerosolization was carried out at a pressure of 1.8 kg/cm^2. The first air sample from the drum was collected after a 15-min period of aerosol stabilization. An impinger containing 10 ml of tryptose phosphate broth with 1% antifoam as aerosol collection fluid was operated for 1 min to draw 5.6 liters of the drum air. Using the same procedure, we collected additional samples of the air at 0.5, 1, 2, 4, 8, 24, 48, 72, and 223 h after virus aerosolization.

The fluid from the impinger was divided into two portions. One of these was used for estimating the amount of the physical tracer and the other was used for virus plaque assay. The extent of biological decay of the virus in the air was calculated by the following formula:

$$\%\ \text{virus survival} = \frac{\text{tracer concentration at time zero}}{\text{tracer concentration at time } t} \times \frac{\text{virus titer at time } t}{\text{virus titer at time zero}} \times 100$$

Survival of the aerosolized virus was tested at the following levels of relative humidity (RH): low (25 ± 5%), medium

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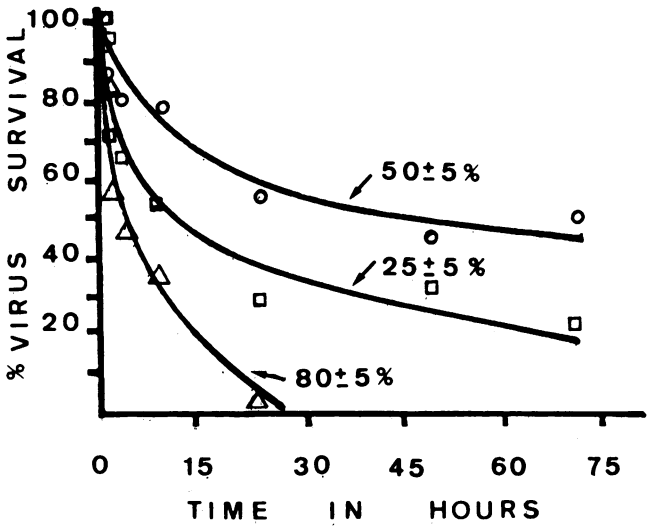

FIG. 1. Effect of relative humidity on the airborne survival of rotavirus SA11 at 20 ± 1°C.

(50 ± 5%), and high (80 ± 5%). For the experiments at the low RH, the drum was first filled with air passed through a Drierite cylinder (Hammond Drierite Co., Xenia, Ohio). Distilled water was sprayed into the drum to raise the RH to the desired level before conducting experiments at the medium and high RH levels.

At least four experiments were conducted at each of the three RH levels. Figure 1 summarizes the findings of these experiments. The virus appeared to survive best at the medium RH at which its half-life was approximately 40 h. At this RH level, about 45% of the infectious virus remained detectable in the drum air even after 72 h of aerosol age. The half-life of the virus at low RH was nearly 9 h, and close to 21% of the infectious virus could be recovered from the drum air 72 h after its aerosolization. High RH was found to be the least favorable to the survival of the aerosolized virus, since 50% of the infectious virus became undetectable within 2 h of aerosolization; at this RH level, no infectious virus could be recovered from the air sample collected 24 h after aerosolization.

To determine how long infectious rotavirus particles remained detectable in the drum air at the midrange RH, a separate experiment was carried out. Nearly 3% of the infectious virus could be detected in the drum air even at 223 h (9 days) of aerosol age.

The findings of this study show that the capacity of rotavirus SA11 to survive in the airborne state (20 ± 1°C) is influenced by RH. In this regard, the RH in the midrange was found to be more favorable compared to the other two RH levels tested. In contrast to this, other nonenveloped viruses such as poliovirus (12), adenovirus (20), and reovirus (1) have been found to survive better at high RH levels. Low levels of RH, on the other hand, have been found to be more conducive to the airborne survival of many types of enveloped viruses (5, 12, 20). Therefore, the behavior of rotavirus SA11, as seen in this study, appears to resemble that of enveloped viruses.

If naturally aerosolized rotaviruses are found to survive in the indoor atmosphere to the same extent, air could readily facilitate their spread in settings such as hospital wards, nursing homes and day-care centers. A number of epidemiological studies on outbreaks of rotaviral diarrhea in certain temperate regions have noted a relationship between the occurrence of such outbreaks and the relatively low indoor RH (2). Data available from tropical areas also indicate that an increase in the number of cases of rotaviral diarrhea in the general community often coincides with periods of cool and dry weather (4, 24, 32).

In many patients in nosocomial outbreaks of rotaviral diarrhea, involvement of the respiratory tract precedes the diarrheal phase (15). Attempts to detect rotaviruses in nasal swabs and washings have, however, been unsuccessful to date (23). Here it should be noted that replication of rotaviruses in the respiratory tract may not be necessary before the production of gastroenteritis; virus-containing aerosols collected in the respiratory tract could be translocated by mucociliary activity and ingested (28). Intranasal instillation of rotavirus-containing inocula has in fact been shown to result in diarrhea in piglets (35) and calves (16).

The observation of Kraft (14) suggested that epizootic diarrhea of infant mice due to murine rotavirus could spread in animal colonies by virus-contaminated air. In other laboratories, spread of rotaviral diarrhea in animal holding facilities is also believed to have occurred by the aerial route (19).

The 1964 outbreak of acute gastroenteritis in a group of islands in the mid-Pacific was shown to be due to a rotavirus with the help of retrospective serological studies (8); the high attack rate an the rapid spread of the outbreak strongly suggested the possibility of aerial spread of the virus.

The role of rotavirus-containing aerosols in the contamination of animate and inanimate surfaces also needs to be considered here. Aerosolized particles of larger size generated during the handling of rotavirus-containing material (33) could lead to the contamination of surfaces in the immediate surroundings. It has already been demonstrated that rotaviruses can survive for prolonged periods on a variety of inanimate surfaces (22).

In addition to the situations described above, generation of aerosols containing rotaviruses could also occur in a number of other settings. Rotaviruses have been detected in domestic sewage (E. M. Smith and C. P. Gerba, Proceedings of the 179th National Meeting, American Chemical Society, **20**:167–169, 1980), and virus-containing aerosols are known to be generated during the treatment and spray irrigation of such wastes (29). Several research and service laboratories now regularly handle rotavirus-contaminated materials. Many routine procedures carried out in such laboratories can lead to the production of infectious aerosols (31).

The work in progress in our laboratory is aimed at studying the airborne survival of rotaviruses from humans and other animals. Such information, together with the findings reported here, should help in understanding the genesis of outbreaks due to rotaviruses and in instituting proper measures for their prevention and control.

This study was supported by grants from the World Health organization and Health and Welfare Canada.

The technical assistance provided by Anton Brunner, Hanne White, Christina Chudzio, and Linda Therrien is gratefully acknowledged. We appreciate the secretarial services of Lina Beaulieu. We also thank A. J. Springthorpe for the computer program used to analyze the data of this study.

LITERATURE CITED

1. **Adams, A. J., J. C. Spendlove, R. S. Spendlove, and B. B. Barnett.** 1982. Aerosol stability of infectious and potentially

infectious reovirus particles. Appl. Environ. Microbiol. **44**:903–908.
2. **Brandt, C. D., H. W. Kim, W. J. Rodriguez, J. O. Arrobio, B. C. Jeffries, E. P. Stallings, C. Lewis, A. J. Miles, R. M. Chanock, A. Z. Kapikian, and R. H. Parrott.** 1983. Pediatric viral gastroenteritis during eight years of study. J. Clin. Microbiol. **10**:71–78.
3. **Cubitt, W. D., and H. Holzel.** 1980. An outbreak of rotavirus infection in a long-stay ward of a geriatric hospital. J. Clin. Pathol. **33**:306–308.
4. **DeTorres, B. V., R. M. DeIlja, and J. Esparza.** 1978. Epidemiological aspects of rotavirus infection in hospitalized Venezuelan children with gastroenteritis. Am. J. Trop. Med. Hyg. **27**:567–572.
5. **Elazhary, M. A. S. Y., and J. B. Derbyshire.** 1977. Effect of temperature, relative humidity and medium on the aerosol stability of infectious bovine rhinotracheitis virus. Can J. Comp. Med. **43**:158–167.
6. **Estes, M. K., E. L. Palmer, and J. F. Obijeski.** 1983. Rotaviruses: a review. Curr. Top. Microbiol. Immunol. **105**:123–184.
7. **Flewett, T. H.** 1982. Clinical features of rotavirus infections, p. 125–145. *In* D. A. J. Tyrrell and A. Z. Kapikian (ed.), Virus infections of the gastrointestinal tract. Marcel Dekker, Inc., New York.
8. **Foster, S. O., E. L. Palmer, G. W. Gary, Jr., M. L. Martin, K. L. Herrmann, P. Beasley, and J. Sampson.** 1980. Gastroenteritis due to rotavirus in an isolated Pacific island group: an epidemic of 3,439 cases. J. Infect. Dis. **141**:32–39.
9. **Goldberg, L. J., H. M. S. Watkins, E. E. Boerke, and M. A. Chatigny.** 1958. The use of a rotating drum for the study of aerosols over extended periods of time. Am. J. Hyg. **68**:85–93.
10. **Halvorsrud, J. and I. Orstavik.** 1980. An epidemic of rotavirus-associated gastroenteritis in a nursing home for the elderly. Scand. J. Infec. Dis. **12**:161–164.
11. **Hara, M., J. Mukoyama, T. Tsuruhara, Y. Ashiwara, Y. Saito, and I. Tagaya.** 1978. Acute gastroenteritis among schoolchildren associated with reovirus-like agent. Am. J. Epidemiol. **107**:161–169.
12. **Harper, G. J.** 1961. Airborne micro-organisms: survival tests with four viruses. J. Hyg. **59**:479–486.
13. **Kapikian, A. Z., H. B. Greenberg, R. G. Wyatt, A. R. Kalica, H. W. Kim, C. D. Brandt, W. J. Rodriguez, R. H. Parrott, and R. M. Chanock.** 1982. Viral gastroenteritis, p. 283–326. *In* A. S. Evans (ed.), Viral infections of humans, 2nd ed. Plenum Publishing Corp., New York.
14. **Kraft, M.** 1958. Observations on the control and natural history of epidemic diarrhea of infant mice (EDIM). Yale J. Biol. Med. **31**:121–137.
15. **Lewis, H. M., H. B. Valman, and D. A. J. Tyrrell.** 1981. Rotavirus infections and respiratory symptoms. J. Pediatr. **98**:511–512.
16. **Light, J. S., and H. L. Hodes.** 1949. Isolation from cases of infantile diarrhea of a filterable agent causing diarrhea in calves. J. Exp. Med. **90**:113–135.
17. **May, K. R.** 1973. The Collison nebulizer: description, performance and application. J. Aerosol. Sci. **4**:235–243.
18. **Middleton, P. J.** 1982. Role of viruses in pediatric gastrointestinal disease and epidemiologic factors, p. 211–225. *In* D. A. J. Tyrrell and A. Z. Kapikian (ed.), Virus infections of the gastrointestinal tract. Marcel Dekker, Inc., New York.
19. **Middleton, P. J., M. Petric, and M. T. Szymanski.** 1975. Propagation of infantile gastroenteritis virus (orbi-group) in conventional and germfree piglets. Infect. Immun. **12**:1276–1280.
20. **Miller, W. S., and M. S. Artenstein.** 1967. Aerosol stability of three acute respiratory disease viruses. Proc. Soc. Exp. Biol. Med. **125**:222–227.
21. **Mitchell, C. A., L. F. Guerin, and J. Robillard.** 1968. Decay of influenza A viruses of human and avian origin. Can. J. Comp. Med. **32**:544–546.
22. **Moe, K., and J. A. Shirley.** 1982. The effects of relative humidity and temperature on the survival of human rotavirus in faeces. Arch. Virol. **72**:179–186.
23. **Nigro, G., and M. Midula.** 1983. Acute laryngitis associated with rotavirus gastroenteritis. J. Infection. **7**:81–83.
24. **Paul, M. O., and E. A. Erinle.** 1982. Influence of humidity on rotavirus prevalence among Nigerian infants and young children with gastroenteritis. J. Clin. Microbiol. **15**:212–215.
25. **Pickering, L. K., D. G. Evans, H. L. Dupont, J. J. Vollet, and D. J. Evans, Jr.** 1981. Diarrhea caused by *Shigella*, rotavirus and *Giardia* in day-care centers: prospective study. J. Pediatr. **99**:51–56.
26. **Ramia, S., and S. A. Sattar.** 1979. Simian rotavirus SA-11 plaque formation in the presence of trypsin. J. Clin. Microbiol. **10**:609–614.
27. **Ramia, S., and S. A. Sattar.** 1980. Concentration of seeded simian rotavirus SA-11 from potable waters by using talc-Celite layers and hydroextraction. Appl. Environ. Microbiol. **39**:493–499.
28. **Slote, L.** 1976. Viral aerosols: a potential occupationally related health threat in aerated wastewater treatment systems. J. Environ. Health **38**:310–314.
29. **Smith, E. M., and C. P. Gerba.** 1980. Survival and detection of rotaviruses in the environment, p. 67–81. *In* S. D. Acres, A. J. Forman, and H. Fast (ed.), Proceedings of the 3rd International Symposium on Neonatal Diarrhea. Veterinary Infectious Diseases Organization, Saskatoon, Saskatchewan, Canada.
30. **Songer, J. R.** 1967. Influence of relative humidity on the survival of some airborne viruses. Appl. Microbiol. **15**:35–42.
31. **Stern, E. L., J. W. Johnson, D. Vesley, M. M. Halbert, L. E. Williams, and P. Blume.** 1974. Aerosol production associated with clinical laboratory procedures. Am. J. Clin. Pathol. **62**:591–600.
32. **Stintzing, G., E. Back, B. Tufvesson, T. Johnsson, T. Wadstrom, and D. Habte.** 1981. Seasonal fluctuations in the occurrence of enterotoxigenic bacteria and rotavirus in paediatric diarrhoea in Addis Ababa. Bull. W.H.O. **59**:67–73.
33. **Totterdell, B. M., I. L. Chrystie, and J. E. Banatvala.** 1976. Rotavirus infection in a maternity unit. Arch. Dis. Child. **51**:924–928.
34. **Tyler, M. E., and E. L. Shipe.** 1959. Bacterial aerosol samples. I. Development and evaluation of the all-glass impinger. Appl. Microbiol. **7**:337–349.
35. **Woode, G. N., J. Bridger, G. A. Hall, J. M. Jones, and G. Jackson.** 1976. The isolation of reovirus-like agents (rotaviruses) from acute gastroenteritis of piglets. J. Med. Microbiol. **9**:203–209.